this increase in the Ca^{2+} spark rate was associated with a decrease in the SR Ca^{2+} content. This is consistent with the increase in the SR Ca^{2+} leak (as evidenced by the increase in the Ca^{2+} spark rate) that followed the H_2O_2 application. Since ROS has been shown to activate other signaling systems in heart (e.g. CaMKII), the interactions between H_2O_2 dependent ROS elevation and both CaMKII and PKA were examined. While significant interactions between rapid, transient ROS elevation and CaMKII and PKA were observed, it was also determined that the actions of these ROS elevations on Ca^{2+} sparks was not mediated by either CaMKII or PKA. How ROS may affect EC coupling under these conditions is also examined and discussed.

Voltage-gated K Channels II

2707-Pos Board B399

Calcium-Activated K Channel Regulates Cell Viability in Hyperkalemic and Hypokalemic Conditions: Implication in the Neuromuscular Disoders

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The involvement of ca-activated-K-(BK) channel in the regulation of cell viability observed under hyperkalemia (15 mEq/L) or hypokalemia (0.55 mEq/L) were investigated in HEK293 cells expressing the hslo subunit (hslo-HEK293) in the presence/absence of BK modulators. The BK openers were: acetazolamide (ACTZ), dichlorphenamide(DCP), methazolamide(MTZ), bendroflumethiazide (BFT), ethoxzolamide(ETX), hydrochlorthiazide(HCT), quercetin(QUERC), resveratrol(RESV) and NS1619; and the BK blockers were: TEA, IbTx and ChTX. Experiments on cell viability and channel currents were performed using cell counting kit-8 and patch-clamp techniques, respectively. Hslo whole-cell current was potentiated by BK channel openers with different potency and efficacy in hslo-HEK293. The efficacy ranking of the openers at −60 mV(Vm) was BFT> ACTZ >DCP ≥RESV≥ ETX> NS1619> MTZ≥ QUERC; HCT was not effective. Cell viability after 24 h of incubation under hyperkalemia was enhanced by 82+6% and 33+7% in hslo-HEK293 cells and HEK293 cells, respectively. IbTx, ChTX and TEA enhanced cell viability in hslo-HEK293. BK openers prevented the enhancement of the cell viability induced by hyperkalemia or IbTx in hslo-HEK293 showing an efficacy which was comparable with that observed as BK openers. BK modulators failed to affect cell currents and viability under hyperkalemia conditions in the absence of hslo subunit. Under hypokalemia cell viability was reduced by −22+4% and −23+6% in hslo-HEK293 and HEK293 cells, respectively; BK channel modulators failed to affect this parameter in these cells. BK channel regulates cell viability under hyperkalemia but not hypokalemia conditions. BFT and ACTZ were the most potent drugs either in activating BK and in preventing the cell proliferation induced by hyperkalemia. These findings may have relevance in disorders associated with abnormal K-ion homeostasis including periodic paralysis and myotonia. Supported by Telethon-GGP10101.

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Stochastic Modeling of Ca^{2+}-Channel / BKCa-Channel Complexes

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Over the last several years evidence has accumulated that indicates that in the brain BKCa-channel activity often affects the firing properties of neurons and in some cases the extent of neurotransmitter release. And it has become clear that BKCa channels often form molecular complexes with voltage-gated Ca^{2+} channels (CaV channels) such that, when a CaV channel is activated, the ensuing influx of Ca^{2+} activates its closely associated BKCa channel. Thus, in modeling the electrical properties of neurons it would be advantageous to have quantitative models of CaV/BKCa complexes. Furthermore, in a population of CaV/BKCa complexes, because all CaV channels are not open at the same time, all BKCa channels are not exposed to the same Ca^{2+} concentration at the same time. Thus stochastic rather than deterministic models are required to simulate the behavior of populations of CaV/BKCa complexes. To date however no such stochastic models have been described. To address this need we have recently developed a stochastic CaV/BKCa model that faithfully reproduces the behaviors of both channels in vitro. Here we describe this model and demonstrate its response to various action-potential-like stimuli. The predicted relationship between the activation of a CaV channel and the subsequent activation of its associated BK channel has been examined. We view our model as an important building block for the development of more complex models of neural function.

2709-Pos Board B401

The Cytochrome C-Like Domain of the Human BK Channel

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The voltage- and calcium-activated BK channel (Slo1) is modulated by small ligands that bind to its intracellular gating ring (GR) formed by four pairs of tandem homologous RCK1 and RCK2 domains. Heme binds reversibly to the GR at site ^{612}CKACH^{616}, a conserved heme regulatory motif (CXXCH) in the Cytochrome C (CytC) protein family, and it is located within the ~120-residue linker connecting RCK1 and RCK2 domains. Most of this linker has thus far evaded structural definition. To gain structural insight on this functionally-significant region, we performed a sequence alignement of BK with CytC and CytC-like domains from different hemoproteins. We found that, in addition to the CXXCH motif, key structural and functional elements of CytC are conserved in the BK RCK1- RCK2 linker: firstly, the portion of the BK region resolved in the available atomic structures shares secondary structure elements with CytC proteins; secondly, CytC positively-charged residues critical for Apaf-1 and cardiolipin interaction align with BK residues K606, K623, R648, K684, K685 and K698; finally, CytC methionine-80, the second axial ligand to the heme iron, aligns with BK M691. These similarities support the premise that a CytC-like domain exists in the BK GR. To experimentally test this hypothesis, we expressed and purified this region (^{598}IAS...LSG^{718}) and probed its structural composition with Circular Dichroism spectroscopy. The α-helical composition of this protein increased following addition of heme (150 nM) from ≈35% to ≈51%, approaching the α-helical content of CytC (≈53%). Moreover, in the full GR, mutation M691A significantly attenuated heme-binding properties as shown by reduced Soret band formation compared to WT, suggesting that M691 is important for heme binding. These results demonstrate that BK channels possess four intracellular CytC-like domains, which may confer novel physiological functions to these ubiquitous ion channels.

2710-Pos Board B402

Enzymatic Activity of the Human BK Channel: A Function Beyond Electrical Signaling

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BK (Slo1) channels open probability is acutely regulated by heme, which associates with their intracellular multi-ligand sensor, the gating ring. We found that the gating ring region encompassing the heme-binding site shares structural homology with Cytochrome C (CytC), the well-known hemoprotein. In addition to its role in electron shuttling, CytC exhibits various catalytic properties such as peroxidase activity, i.e. the oxidation of suitable substrates using peroxides. To probe for peroxidase activity of the CytC-like domain in a purified BK channel gating ring, we used the chromophore 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as the oxidizable substrate. We found that the BK gating ring complexed with heme in the presence of H_2O_2 catalyzes the formation of $ABTS^{+\bullet}$ cation radical, monitored by absorption at 415nm. The initial rate of $ABTS^{+\bullet}$ formation was linearly correlated with [gating ring] between 0.05-0.3 μM. Disruption of the heme regulatory motif (C615S/H616R) significantly decreased the initial rate of $ABTS^{+\bullet}$ formation. The kinetic parameters of the enzymatic reaction were determined by performing two-substrate Michaelis-Menten analysis, which yielded for gating ring: $k_{cat}/K_m^{H2O2} \approx 12\ s^{-1}mM^{-1}$ and $k_{cat}/K_m^{ABTS} \approx 0.5\ s^{-1}mM^{-1}$. For CytC, we estimated $k_{cat}/K_m^{H2O2} \approx 1.4\ s^{-1}mM^{-1}$ and $k_{cat}/K_m^{ABTS} \approx 0.035\ s^{-1}mM^{-1}$. These results suggest that, under our experimental conditions, the gating ring catalytic efficiency is ~10 times higher than CytC. Finally, we found that HEK cells expressing BK channels (blocked with 100 nM Iberiotoxin) are significantly more resistant to oxidative insult (200 μM H_2O_2) than cells expressing BK channels with impared heme binding (C615S/H616R) ($p<0.05$) as revealed by the increased cell viability (MTT assay). Thus, the BK channel exhibits peroxidase activity and confers a protective effect against oxidative cell damage. These results redefine the role of BK channels, assigning a catalytic property, in addition to their established K^+ conducting properties.

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BK β1 Transmembrane Regions Critically Control the Characteristic Phenotype of β1-Containing Bk Channel Currents

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